Chapter

Small Molecule/HLA Complexes Alter the Cellular Proteomic Content

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Abstract

A medical product usually undergoes several clinical trials, including the testing of volunteers. Nevertheless, genomic variances in the patients cannot be considered comprehensively and adverse drug reactions (ADRs) are missed or misinterpreted during trials. Despite the relation between ADRs and human leukocyte antigen (HLA) molecules being known for several years, the fundamental molecular mechanisms leading to the development of such an ADR often remains only vaguely solved. The analysis of the peptidome can reveal changes in peptide presentation post-drug treatment and explain, for example, the severe cutaneous ADR in HLA-B*57:01-positive patients treated with the antiretroviral drug abacavir in anti-HIV therapy. However, as seen in the biophysical features of HLA-A*31:01-presented peptides, treatment with the anticonvulsant carbamazepine only induces minor changes. Since the binding of a drug to a certain HLA allelic variant is extremely distinct, the influence of the small molecule/protein complex on the proteomic content of a cell becomes clear. A sophisticated methodology elucidating the impact of drug treatment on cells is a full proteome analysis. The principal component analysis of abacavir, carbamazepine or carbamazepine-10,11-epoxid treated cells reveals clear clustering of the drug-treated and the untreated samples that express the respective HLA molecule. Following drug treatment, several proteins were shown to be significantly up- or downregulated. Proteomics and peptidomics are valuable tools to differential clinical outcomes of patients with the same HLA phenotype.

Keywords: Adverse drug reaction, human leukocyte antigen, abacavir, carbamazepine, proteome

1. Introduction

Since treatment with drugs can trigger harmful adverse events, several tests have to be performed before the approval of new drugs. In preclinical trials, the substance is tested in cell culture or animal experiments in order to ascertain its pharmacokinetics, the pharmacodynamics and to exclude any toxic effects. Clinical trials are designed for the examination of the efficacy and safety of a drug under

defined parameters; they are differentiated into different stages [1]. Clinical trials can be randomized, masked, placebo-controlled or crossover studies. Therefore, they are favored towards non-interventional case–control studies [2].

Phase 0 studies are first-in-human-studies using subtherapeutic dosage of the tested drug in a small group of fewer than 15 healthy volunteers to asses pharmacokinetics and pharmacodynamics [3]. In phase I studies therapeutic dosages of the drug are tested in healthy volunteers to examine its tolerability and safety [4]. They are not randomized trials, making them susceptible for selection bias [5]. Phase II studies are more broadly conceived, and the drug is tested in sick individuals for spotting its efficacy, optimal doses and tolerability, including potential side effects. This same occurs in phase III studies where several thousands of volunteers are tested in order to prove a significant therapeutic effect of the drug under study. After drug's approval, the pharmacovigilance can still be monitored in the so-called post-marketing surveillance trials or phase IV studies [3].

Despite these different stages of testing, genomic variances in the patients cannot be considered completely. While differences in metabolism are easier to spot, there are other genes not being taken into account, thus leading to the lack of some adverse drug reactions (ADRs) in clinical trials [6].

2. Adverse drug reactions (ADRs)

2.1 ADRs as an underestimated factor in the health care system

If harm is occurring during treatment with a drug, it can be termed as an adverse event (AE), regardless of a causal link between the drug usage and the symptoms. However, adverse drug events (ADEs) are caused by the drug application [7–9]. This includes harm triggered by the substance itself, as well as harm induced by inappropriate dosages or premature discontinuation of the medication [7, 10]. For example, the overdose of a drug is an ADE. Depending on the drug, the probability of occurring an ADE differs, being very low in patients treated with for instance with antimitotics, and very high in patients under immunosuppressive medication [11]. This is explicable by the mode of action of the drug, for instance the antimitotic nystatin attaches to the cell membrane of fungal cells causing their disruption, but does not disrupt human cells. Contrarily, immunosuppressive medication may led a patients to be more prone to infections and cancer, since the whole immune system is suppressed [10].

Despite a correct dosage and application, unintended and harmful reactions to drugs can still occur [12]. Such ADRs are distinguished from side effects that comprise positive, negative and irrelevant unintended effects [7, 10, 11].

ADRs can be classified into dose-dependent and predictable type A and dose-independent idiosyncratic type B [13] (see **Figure 1**). Most ADRs are type A reactions (>80%), explicable by the pharmacological activity of the drug [13, 14]. Therefore, they can occur in nearly all patients [14]. Type A ADRs are rarely fatal, and the symptoms are drug-specific [13–15]. These ADRs are affected by drug pharmacokinetics, comorbidities and drug–drug-interactions [14]. In contrast to type B ADRs, the emergence of type A ADRs are comprehensible [10]. At the first appearance, type B ADRs seem to be idiosyncratic, but the immune system is often involved and, in these cases, they are called drug hypersensitivity reactions [10]. The clinical picture can involve a single organ or be systemic [16]. Despite their less frequent occurrence, type B ADRs are characterized by an increased mortality rate [13, 14].

Type B ADRs can be subclassified depending on the drug's mode of action with immune cells into allergic, pharmacologic and pseudoallergic reactions [14].

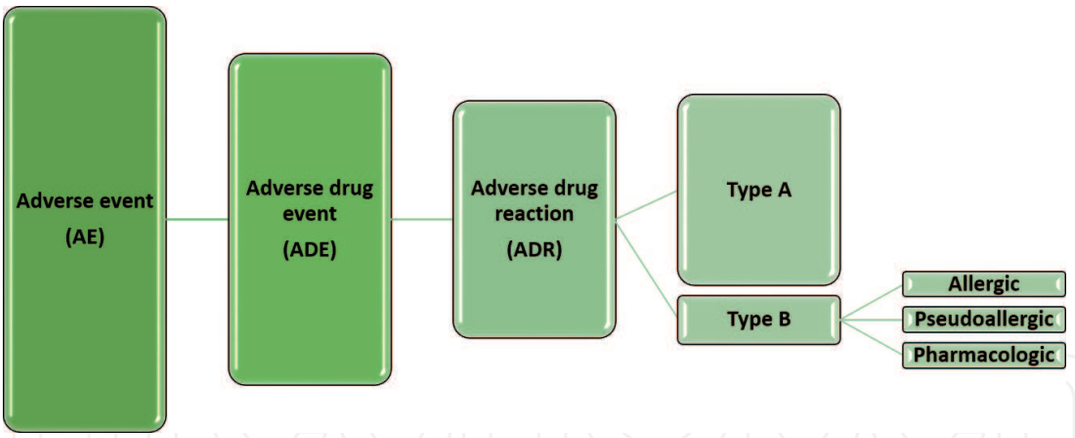

Figure 1.
Classification of ADRs. ADRs are ADEs that occur despite a proper dosage and application, and are mainly subclassified into type A and rare type B reactions.

Hereby, allergic reactions are mediated by both the innate, as well as by the adaptive immune systems and include, for instance, the IgE-mediated penicillin allergy or contact dermatitis. Pseudoallergic reactions manifest, for example, as urticaria/anaphylaxis bronchospasm. Pharmacologic reactions are T cell-mediated. Other possible classifications are relative to the time point of the first symptoms, or to their type of immune mechanism or type of drug [14].

The ADRs have often arisen as an underestimated factor in the health care system, due to their underreporting and underdiagnosis [15, 17–20].

2.2 Type B ADRs manifest as different clinical pictures

Type B ADRs can be systemic or affect certain organs, with skin, liver and blood cells being the most impacted [16]. Cutaneous forms of ADRs include, for example, maculopapular exanthema (MPE), acute generalized exanthematous pustulosis (AGEP), drug reaction with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [10].

The MPE is relatively mild, forming rashes with macules or erythematous and maculopapular lesions [21, 22].

AGEP is known to have an acute onset characterized by fever, large erythema and sterile, non-follicular pinhead-sized rapidly appearing pustules with desquamation starting from four to ten days later [10, 23]. The mucosa is barely involved; other organs are free of symptoms. Several drugs are shown to induce AGEP, among which we can find aminopenicillins, quinolones and pristinamycine [10, 23].

The DRESS, also known as drug-induced delayed multiple organ hypersensitivity syndrome (DIDMOHS), drug-induced hypersensitivity syndrome (DIHS), drug hypersensitivity syndrome (DHS) or hypersensitivity syndrome (HSS), is characterized by a cutaneous exanthema spread for over more than half of the body and other organ's involvement, such as hepatitis, eosinophilia, arthralgia or lymphadenopathy [10, 24]. DRESS can be triggered by anticonvulsants (carbamazepine (CBZ), oxcarbazepine, lamotrigine, phenytoin and phenobarbital), sulfonamides and uricostatic drugs (allopurinol) [24].

Although overall SJS and TEN may be fatal in 20–25% of all cases, in TEN the mortality may increase up to 48% and, in the elderly, TEN can be fatal in 70% [24, 25]. Typically, SJS/TEN manifest with skin blisters and bullae, detachment of the skin and erosions of mucous membranes [26]. In SJS, up to 10% of the body surface area is affected, while in TEN at least 30% is affected; if between 10% and 30% of the body surface area are affected a transitional form is diagnosed [25].

Additionally, complications with the lungs, fever and hypovolemia may occur [25]. It has been shown that apoptotic signal-associated cytokine levels are increased in SJS/TEN [27]. Patients suffering from SJS/TEN are positive for Nikolsky's sign, yet specific laboratory parameters are still lacking [28].

The algorithm of drug causality for epidermal necrolysis algorithm (ALDEN) is designed to ascertain the correct diagnosis [29]. SJS/TEN can be triggered not only by anticonvulsive medication (CBZ, lamotrigine, phenytoin and phenobarbital), sulfonamides and uricostatic drugs (allopurinol), but also by oxicam-NSAIDs, sulfasalazine and antiretroviral medication (nevirapine) [30].

3. Associations of human leukocyte antigen (HLA) alleles with type B ADRs

Associations of certain alleles of the human leukocyte antigen (HLA) system with type B ADRs have been previously reported [31]. The HLA molecules are cell surface glycoproteins that present peptides to immune cells exerting their crucial function in the recognition of self/non-self. By varying in their function and structure, HLA class I and II molecules can be differentiated. Whereas HLA class II molecules are composed of two membrane-anchored chains α and β, HLA class I molecules are composed of the invariant light chain β_2-microglobulin (β_2m) non-covalently linked to the membrane-anchored heavy α-chain [32]. The peptide-binding groove of the HLA class I molecules is formed by the α1 and α2 domains, where a peptide with a length of eight to ten amino acids is presented. Contrarily, HLA class II molecules present longer peptides, since their peptide-binding groove formed by the α1 and the ß1 domains is open in both ends. HLA class I molecules interact with $CD8^+$ T cells and present peptides of intracellular origin, whereby HLA class II molecules display peptides derived from the extracellular space or from vesicles to interact with $CD4^+$ T cells [33]. As part of the adaptive immune system, T cells are able to scan cells for the presence of antigens inducing the death of the respective cells, or releasing of cytokines leading to the activation of other immune cells. Some differences can also be found in the expression patterns of HLA molecules. While HLA class I is expressed by all nucleated cells and platelets, HLA class II expression is limited to immune cells, such as antigen presenting cells, macrophages and B cells [32, 34].

The HLA molecules are characterized by an exceptional polygenism and polymorphism [35]. The HLA genes are encoded in a 220-genes-encompassing region organized in HLA class I, class II and class III genes, whereby class III genes are immune system-related [32, 35, 36]. Among the currently known 28,786 alleles, 20,597 are HLA class I alleles and 7,723 are HLA class II alleles, making it impossible to consider allelic variants in clinical trials [35]. Therefore, HLA-mediated ADRs are inevitably overlooked before the approval of the drug.

As such, the association of abacavir (ABC) hypersensitivity with HLA-B*57:01 is the best known [37]. About 5% of HIV patients that are treated with ABC show symptoms [38, 39]. Other examples are the association of CBZ hypersensitivity with two alleles, HLA-A*31:01 and HLA-B*15:02, and of allopurinol hypersensitivity with HLA-B*58:01 [22, 40, 41]. In Han Chinese, all patients developing CBZ hypersensitivity were positive for HLA-B*15:02 [41]. In Northern Europeans, in the presence of the allele HLA-A*31:01, the risk for an ADR increases from 5–26%, whereas in its absence it decreases to 3.8% [22]. Moreover, the association of ticlopidine, nevirapine and/or dapsone hypersensitivity with the alleles HLA-A*33:03, HLA-DRB1*01:01 and HLA-B*13:01 has also been described [42–44] (see **Figure 2**). In general, ADRs can occur in about 15% of the patients during hospitalization [52].

Figure 2.
Depiction of some HLA-associated ADRs. Each example box includes the name of the drug, the associated HLA allele, the author of the first publication, the journal and year of the publication, the syndromes and adverse reactions (SJS/TEN in orange, MPE and DRESS in yellow, hepatotoxicity/drug-induced liver toxicity in green, mixed symptoms in light orange) and the population where the association was observed. Among others the following drugs were shown to be associated with ADRs: Abacavir [37, 45], carbamazepine [41, 46], allopurinol [40], nevirapine [44], phenytoin [47], sulfamethoxazole [48], ticlopidine [42], flucloxacillin [49], lamotrigine [50], oxcarbazepine [51], dapsone [43].

3.1 Peptide loading of HLA class I molecules

Peptide loading occurs in the endoplasmic reticulum after biosynthesis and folding of the nascent HLA class I heavy chain. The interaction with the chaperone

calnexin stabilizes the association of the HLA class I heavy chain with the light chain β_2m [53]. The peptide loading complex (PLC) is also composed of the chaperone calreticulin, the transmembrane glycoprotein tapasin and the thiol oxidoreductase endoplasmic reticulum resident protein 57, which ensure the correct glycosylation, folding and peptide loading [54].

Peptides presented by HLA class I molecules derive from the cytosol. In the cytosol, ubiquitinylated proteins are degraded via proteasomes into short peptides with a length of 3–22 amino acids [32, 55]. The transporter associated with antigen processing subserves ATP-dependent translocation of cytosolic peptides into the lumen of the endoplasmic reticulum, where they are loaded onto the HLA class I molecule [32]. Ubiquitinylated proteins are composed of misfolded and aged proteins, together with defective ribosomal products, that comprise up to 30% of the newly synthesized proteins [56, 57]. Thereby, a rapid $CD8^+$ T cell reaction is enabled in infections [58].

3.2 Peptide presentation by HLA class I molecules

As already described above, the peptide binding region (PBR) is shaped by the α1 and α2 regions of HLA class I molecules, and the α1 and ß1 regions of HLA class II molecules. What they have in common is the basic structure composed of a β-sheet at the ground of the PBR, and two α-helices that form the sidewise boundaries [32].

Solely those peptides with a certain amino acid sequence fit into the PBR of an HLA allele. HLA alleles mostly differ in the PBR region, which gives them a unique peptide binding motif, since alterations in the shape of the PBR lead to the presentation of an altered set of peptides. The PBR of HLA class I molecules is partitioned into pockets A-F, with pocket A binding residue 1 of a given peptide, pocket B binding residue 2 and so on [36, 59–61]. Typically, a peptide binding motif is defined by a N- and a C-terminal anchor, the amino acids at p2 and pΩ binding to pocket B and F [32, 61]. The side chains of the presented peptide can bind either into the pockets or point outwards. This complex of the peptide and HLA molecule is scanned by T cells that are able to recognize foreign peptides in the complex of self HLA.

4. Activation of the adaptive immune system by drugs

During the maturation of T cells, positive and negative selection assure the generation of an HLA-restricted, but self-tolerant, T cell receptor (TCR) repertoire. Therefore, viral, bacterial or stress-related peptides present in case of infection are recognized by the immune system when $CD8^+$ and $CD4^+$ T cells scan the HLA molecules. The TCR is composed of two chains, α and β, with each obtaining three complementarity determining regions (CDRs) named CDR1, CDR2 and CDR3. These are extremely variable loops able to recognize both the combination of the HLA molecule and the peptide [32].

For the activation of $CD8^+$ T cells, not only the interaction of the TCR is necessary, but also the interaction of the CD8 co-receptor with the HLA molecule, leading to the phosphorylation of the immunoreceptor tyrosine-based activation motifs [62, 63]. As a second signal, the CD28 molecules on naïve T cells need to interact with a receptor of the B7 family on the target cell, aiming to ensure their survival. Finally, cytokines initiate the third signal by triggering the clonal expansion and differentiation into effector cells. The activated cytotoxic T cell can cause the apoptosis of the target cell through the release of granules with perforin, granzymes, and a scaffold protein triggering the activation of the caspase 3 [64].

Synthetical drugs usually only have a size of less than one kDa, making them invisible to the immune system. Nevertheless, they can induce a reaction within the immune system through their binding to a carrier protein (hapten), or after metabolization of the drug (prohapten) [21]. This hapten-protein complex can trigger several immune reactions, from type I to IV, according to Gell and Coombs [14]. The binding of IgE antibodies to the complex activates mastcells and basophils in type I reactions [14, 21]. This can be seen, for example, in allergy caused by β-lactam antibiotics manifesting as urticarial and anaphylaxis [14]. Type II reactions are mediated by IgG and IgM antibody-dependent cell-mediated cytotoxicity, and are seen in aminopyrine hypersensitivity leading to leukopenia. On the other hand, type III reactions are characterized by IgG-driven immune-complexes that are deposited or cleared by complement activation [10, 14, 21]. A type III reaction example is the minocycline-mediated DRESS [10]. Delayed type IV reactions are generally triggered by T cells.

T cells have been isolated from the blister fluid of patients suffering from cutaneous ADRs [65, 66]. Three models (1, 2 and 3) tend to explain the involvement of cytotoxic T cells in HLA class I-associated ADRs.

1. In the first model, called the hapten/prohapten model, the drug or its metabolite can bind as hapten either to a peptide that is later presented in the context of self-HLA, or to a protein that is processed and subsequently presented as a modified self-peptide [67–71]. This has been shown in cases of allergy to β-lactam antibiotics that are B and T cell-mediated; the hapten binds to lysine side chains of presented peptides [72–75]. Moreover, T cell proliferation and toxicity are induced by a reactive nitroso metabolite of the antibiotic sulfamethoxazole, which is able to bind to peptides that are presented in the context of self-HLA [76].

2. The second model is the pharmacological-interaction (p-i) model that initiates the fast and direct activation of cytotoxic T cells, independently from the metabolism and peptide processing, by a noncovalent interaction of the drug with the HLA molecule and/or the TCR. The reversible and potentially weak interaction established between the drug and the immune receptors induces functional changes in the conformation of the immune receptors [77, 78]. In the case of allergies caused by sulfamethoxazole, the p-I models can also be applied, since T cells can be stimulated with fixed sulfamethoxazole-treated cells, being possible to wash the drug off afterwards [79, 80].

3. In the third model, the altered repertoire model, the binding of the drug to the PBR of the HLA molecule induces an alteration in its shape and ability to present peptides, so that an altered peptide repertoire is selected and recognized as foreign [81, 82]. This is seen in ABC hypersensitivity, where the drug binds to the F pocket of HLA-B*57:01, thus triggering an alteration in the pΩ anchor [83, 84].

5. Analysis of the peptidome in HLA-associated ADRs

The drug ABC is a guanosine-analogue indicated for HIV therapy. ABC hypersensitivity manifests as a systemic disease, striking up 11 days upon start of the treatment [37]. Fever, rash, constitutional symptoms, and gastrointestinal symptoms, such as nausea, vomiting, diarrhea, or abdominal pain, characterize the clinical picture of ABC hypersensitivity [37, 39]. In 2002, its association with

HLA-B*57:01 has been published [37, 45] and in 2008, the testing for the presence of HLA-B*57:01 in patients was recommended to reduce the risk of ABC hypersensitivity [39].

In order to prove or disprove the altered repertoire model, analysis of the peptidome has been performed. Furthermore, it is also possible to unravel the structure of the drug bound to the HLA molecule, by using a peptide found in the analysis of the peptidome. In ABC hypersensitivity, both experiments were performed. The crystal structure of ABC bound to the F pocket of HLA-B*57:01 has shown that this position is already occupied by the drug, thus leading to an alteration in the peptidome [83]. Typically, peptides presented by HLA-B*57:01 are anchored by a C-terminal tryptophan, tyrosine or phenylalanine [83, 84]. The alteration in the chemical properties of the PBR enables binding of a new repertoire of endogenous self-peptides [83, 84]. These peptides will then trigger the activation of ABC-specific cytotoxic T cells, resulting in ABC hypersensitivity [83].

The drug CBZ is a tricyclic anticonvulsant usually used in the therapy of bipolar disorders, as well as in nerve pain [21, 85–88]. Certain patients treated with CBZ can develop severe SJS/TEN, DRESS or MPE, as recognized soon after the approval of the drug [86, 89]. Later on, the association of CBZ-mediated SJS/TEN with HLA-B*15:02 became evident, primarily in South East Asian populations [41, 90–92]. Interestingly, in Caucasians and some Asian populations, milder symptoms, such as DRESS and MPE, were found to be associated with HLA-A*31:01 [22, 81, 93, 94]. As the allele HLA-B*15:02 is mostly found in South Asia, being nearly absent in Europe [95], this may explain why the HLA-B*15:02 is not found in Caucasians with CBZ hypersensitivity [96]. Contrarily, the allele HLA-A*31:01 has been shown to be distributed worldwide [95]. Despite clearly differing in their sequence in the PBR, both alleles are associated with CBZ hypersensitivity [21]. However, research has shown that CBZ hypersensitivity is presented as two distinct diseases forms with differing mechanisms of action [21], consistent with the different clinical pictures and median onset of HLA-B*15:02- and HLA-A*31:01-associated CBZ hypersensitivity [24, 95].

The altered repertoire model has been discussed for the association of HLA-B*15:02 with CBZ-mediated SJS/TEN, but no clear alterations in the peptidome, after treatment with CBZ, were found [83, 97, 98]. Additionally, derivates of CBZ have been shown to bind soluble immobilized HLA-B*15:02 [99]. Later published studies have revealed that the main metabolite CBZ-10,11-epoxide (EPX) was binding to the F pocket, so that the nonpolar aromatic pΩ anchor was no longer able to bind that position [81]. These findings are in agreement with those where a polymorphism in the epoxide hydroxylase 1 was influencing the risk of SJS/TEN in the Han Chinese population [100]. Nevertheless, this does not explain the activation of CBZ-specific T cells *in vitro* [99, 101].

In HLA-A*31:01-associated CBZ-mediated ADRs, no clear alterations in the peptide binding motif post CBZ and EPX treatment have been found [21].

6. Analysis of the proteome in HLA-associated ADRs

The field of proteomics greatly contributes to a comprehensive profiling of the immune response. To enable side effect predictions for uncharacterized drugs, and to prevent the delay in the licensing process, one widely used action is the analysis of drug (small molecule)-protein interactions [102].

Small molecules-targeted proteins [103] are clearly disturbed or even enabled on their protein–protein-interaction networks. The ability of a protein to initiate the onset of expression, regulation, and/or function of its cognate interaction

partner, highly depends on its structural integrity. Drugs are not only physically, but also functionally, involved with many other proteins and cellular components, as both drugs and proteins are embedded in cellular pathways and networks [103]. The identification of regulated proteins following drug treatment provides insight into the regulatory impact of drugs on target cells (see **Figure 3**). The classical HLA-Ia molecules, one of the molecular interaction partners of small molecules, are genetically very polymorphic and structurally highly variable. This variability is attributed to the peptide repertoire that can be intracellularly selected and extracellularly presented by the distinct HLA-Ia molecules. This structural variability

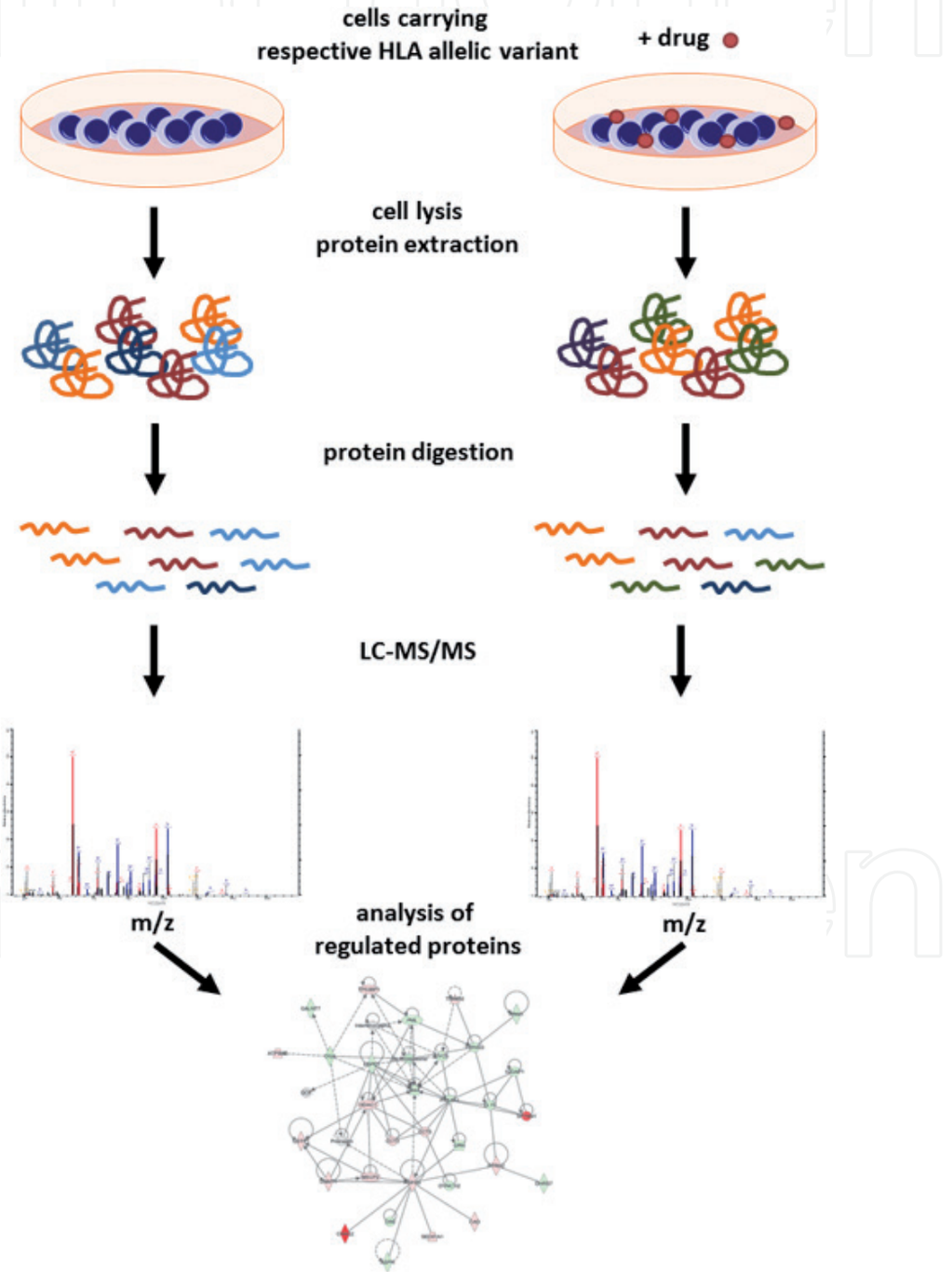

Figure 3.
Workflow of protein drug profiling. Comprehensive analysis of protein abundance in drug-treated cells compared to control cells. After drug treatment, the cells were lysed, and proteins extracted from the sample. Proteins were digested into peptides and analyzed by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). Significant regulated proteins were determined and analyzed via pathway analysis.

makes molecular and bioinformatical analyses of drug-HLA interaction calculations impossible. Unfortunately, the binding of a drug to an HLA-Ia molecule has a profound impact on certain HLA-allele carriers [97]. To somehow enable bioinformatic calculations, experimental achievements in the analysis of small molecule-protein interactions showed broad alterations in the proteome repertoire of targeted cells [21]. Proteomic analysis provides information on protein expression, and mass spectrometry (MS)-based protein drug profiling, improves the understanding of presentable peptides and identification of HLA-bound ligands [104].

ABC-mediated ADRs in HLA-B*57:01 positive individuals are unique in their rapid emergence [105]. Although it could be possible to demonstrate that ABC alters the chemical properties of the PBR, and elicits immune responses through ABC-specific T cells, not all HLA-B*57:01 positive individuals develop ABC-induced hypersensitivity reactions [39]. It becomes obvious that not only the HLA type, but also further individual patient-specific factors, may contribute to ABC-mediated ADRs. The proteome analysis of ABC-treated cells provides insights into the regulatory impact of ABC in the HLA-B*57:01-expressing cells (see **Figure 4**). ABC treatment resulted in an increased apoptosis rate; proteins that generally lead to decreased viral replication were differentially regulated, such as PML and TNPO3. Furthermore, ABC treatment provided hints towards an increased proteasomal degradation activity that would enlarge the pool of presentable peptides. The proteomic drug profiling of ABC-treated cells allowed to enlarge the knowledge about ABC-dependent cellular changes.

CBZ-mediated ADRs are associated with HLA-A*31:01 and HLA-B*15:02. A recent study about HLA-B*15:02-restricted CBZ-induced ADRs revealed that EPX, as the main metabolite, might be responsible for severe reactions in HLA-B*15:02-positive individuals [81, 99]. To increase the understanding of differential clinical courses, proteome analysis of CBZ- and EPX-treated cells has been performed [21]. CBZ treatment of HLA-A*31:01-positive cells provided evidence towards an increased ubiquitination activity, but with a stable cellular viability. On the other hand, EPX treatment of HLA-B*15:02-positive cells resulted in increased cytokine

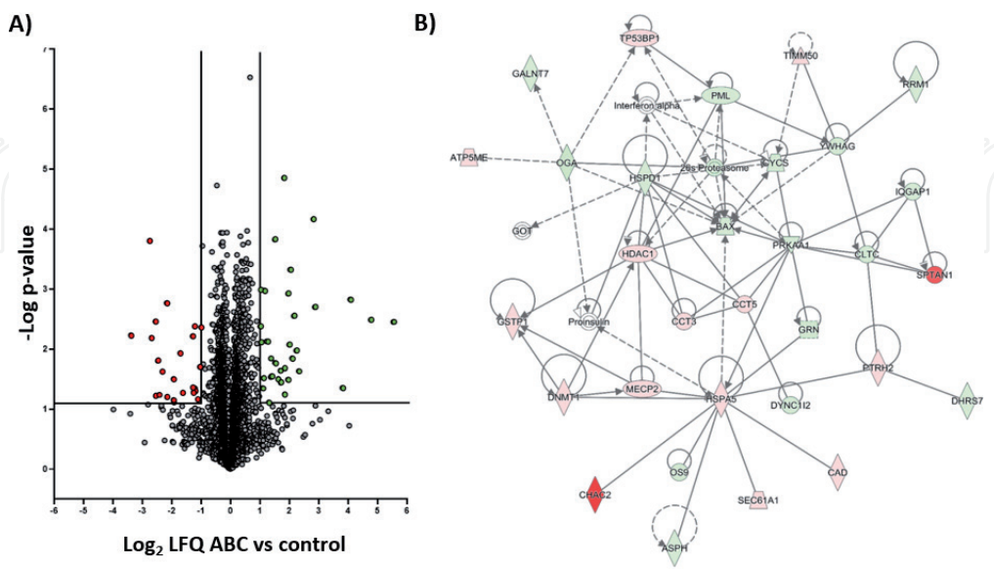

Figure 4.
Mass spectrometric analysis of the proteome of Abacavir (ABC) treated and non-treated cells. A) Protein abundance after ABC treatment. Significantly upregulated proteins are shown in green and downregulated proteins are shown in red. B) Network analysis for up- and downregulated protein groups following ABC treatment. Upregulated proteins are illustrated in red, downregulated proteins are illustrated in green; non-colored proteins were added by the IPA algorithm. High confident interactions are represented by a continuous line; medium confident interactions are represented by a dashed line.

release [21]. The proteomic analyses of CBZ and EPX-treated cells provided the first perceptions into the potential protein regulation and involvement of cellular pathways. Furthermore, proteomic profiling has also shown to contribute to the comprehensive understanding of CBZ-induced ADRs in the context of HLA specificity.

A deep knowledge over the spectrum of proteins that are influenced by drug/protein complexes clearly plays an important role in drug safety, and offers the possibility to identify potential toxicity targets. The emerging role of proteomics improves personalization of immunotherapy treatment in HLA- associated diseases, since detail target analysis supports the understanding of enigmatic HLA-associated ADRs.

7. Conclusions

The proteomic repertoire is a real time view on the health status of a cell, and can be altered through the medical condition of the illness after treatment with the respective drug. Therefore, the knowledge of the proteomic repertoire of a healthy cell pre- and post-treatment with a given drug is indispensable and should not be underestimated.

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Conflict of interest

The authors declare no conflict of interest.

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